

### **Amendments to the Specification**

Please add the priority information paragraph to the specification by inserting the following new paragraph before the first line of the specification:

This application is a 371 of International Patent Application No. PCT/EP03/008569, filed 31 July 2003.

An Abstract on a separate sheet is attached as required under 37 CFR 1.72(b). Please insert the attached abstract, following the claims.

Please add the following paragraph on page 4, at line 19:

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 illustrates a schematic of L1, L2, L3 and L4 immunotypes.

Fig. 2 illustrates a schematic of L3 and L2 immunotypes (H44/76, MC58 strains).

Fig. 3 illustrates a schematic of L3 and L1 immunotypes

Fig. 4 provides DNA sequences of 35E (SEQ ID NO: 12) and lgtGF (SEQ ID NO: 13).

Please replace paragraph 3, beginning on page 24, with the following amended paragraph:

In order to fix the expression of the *lgtA* gene so that it was fixed "on" we altered the homopolymeric tract of the *lgtA* gene so that only 2 G residues remained in the homopolymeric tract region (the wild type strain, MC58, has 14 G; Jennings *et al* 1995, *supra*). Using primers Lic31ext: 5'- CCT TTA GTC AGC GTA TTG ATT TGC G -3' (SEQ ID NO:1) and lgtAG2 5'-ATC GGT GCG CGC AAT ATA TTC CGA CTT TGC CAA TTC ATC - 3' (SEQ ID NO:2) in PCR with *Neisseria meningitidis* strain MC58 chromosomal DNA as template we amplified the region to be altered. The latter primer incorporated the change in the *lgtA* sequence from 14G to 2G. The resulting PCR product was cloned into pT7Blue (Novogen), to create plasmid pT7lgtAG2. To reconstitute to complete *lgtA* gene so that the plasmid could be used to transform the new allele into *Neisseria meningitidis*, a *Bss*HIII fragment from plasmid p1B11 (Jennings *et al* 1995, *supra*) was cloned into the *Bss*HIII site of

pT7lgtAG2 in the correct orientation. Nucleotide sequence analysis confirmed the correct orientation of the gene and that the sequence segment was identical to the corresponding section of the wild-type *lgtA* gene (Genbank accession NMU25839) apart from the alteration of the homopolymeric tract from 14 to 2 G residues. Using a similar process, variants of the lgtAG2 primer mutations were made so that a series of similar plasmids were created that contained *lgtA* alleles with 3, 4, 5, 7 and 10 G residues in the homopolymeric tract region.

Please replace paragraph 2, beginning on page 25, with the following amended paragraph:

In order to transfer the *lgtAG2* mutation to the chromosome of *Neisseria meningitidis* to make a mutant strain, the plasmid pT7lgtAG2 was linearized and used to transform *Neisseria meningitidis* strain MC58 $\phi$ 3 containing an *lgtA::kan* mutation (Jennings *et al* 1995, *supra*). Positive colonies were detected by mAb 4A8B2 in colony-immunoblot (Jennings *et al* 1999, *supra*). Confirmation that the LgtA positive phenotype (L3 immunotype structure) of the transformants was the result of the transfer of the *lgtAG2* allele to the chromosome was confirmed by PCR of the relevant section of the *lgtA* gene using primers Lic31 ext and Lic16ext: 5'- CGA TGA TGC TGC GGT CTT TTT CCA T -3' (SEQ ID NO:3), followed by nucleotide sequencing with the same set of primers. The resulting strain 2G2 had the genotype: MC58 parent strain; *siaD::ery lgtAG2*). Strain 2G2 was subsequently transformed with the a plasmid containing an *lgtB::kan* mutation (Jennings *et al* 1995, *supra*) to create strain 2G2ecoNI, this strain had the genotype: MC58 parent strain; *siaD::ery lgtAG2 lgtB::kan*

Please replace paragraph 3, beginning on page 27, with the following amended paragraph:

Using strain *Neisseria meningitidis* strain 35E (L2 immunotype typing strain) as a template primer pair Lg1: 5'-ATG AAG CTC AAA ATA GAC ATT G-3' (SEQ ID NO:4) and Lg21: 5'- ATC TGC GGG CGG CGG CGC GAC TTG GAT-3' (SEQ ID NO:5), and primer pair LGdel18: 5'-GAA TTC GGA TCC AAC TGA TTG TGG CGC ATT CC-3' (SEQ ID NO:6) and Lg2UP: 5'-TGC CGT CTG AAG ACT TCA GAC GGC TTA TAC GGA TGC CAG CAT GTC-3' (SEQ ID NO:7) (underlined

sequence denotes a *Neisseria meningitidis* uptake sequence) were used to produce two PCR products. These products were purified and then used in splice overlap PCR with primers Lg 1 and Lg2UP to produce a final product that was cloned into the pGEM-T Easy vector (Promega). The resulting plasmid, pL2+, was sequenced to confirm that the wild type sequence of 11C in the wild type polyC tract of *lgtG* had been replaced with 5'-CGCCGCCGCC-3' (SEQ ID NO:8). The sequence of the *lgtG* coding sequence in the region of the mutation is shown in figure 4 [which shows the alignment of nucleotide sequence of the wild-type sequence of the *lgtG* gene of *Neisseria meningitidis* strain 35E and the *lgtG* "fixed" mutation (underlined, bold) contained on plasmid pL2+. Also shown is an *XcmI* restriction endonuclease cleavage site used to construct an *lgtG::kan* mutant].

Please replace paragraph 1, beginning on page 28, with the following amended paragraph:

In order to transform the *lgtG* "fixed" mutation and detect the LPS phenotype with immunocolony-blot screening it was necessary to create a strain that was fixed "off" expression for *LgtG*. A kanamycin cassette from pUK4kan was clones into the *XcmI* site of pL2+. The resulting plasmid, plgtG::kan, was used to transform 2G2 (see above) to kanamycin resistance and the correction position of the *lgtG::kan* allele was confirmed by PCR using primers Lg1 and Lg4 5'-AACCGTTTTCCTATTCCCAT-3' (SEQ ID NO:9), followed by nucleotide sequencing with the same primers. The resulting strain,  $\phi$ 3lgtA2GlgtG::kan-3, had the genotype: MC58 parent strain; *siaD::ery lgtAG2 lgtG::kan*. This strain was then transformed with plasmid pL2+ and screened for colonies with an L2 phenotype and screen by colony-immuno blots (Mn 42F12.32). Positive colonies were picked and tested for by both kanamycin sensitivity and PCR using primers Lg1 and Lg8 5'-CAC CGA TAT GCC CGA ACT CTA-3' (SEQ ID NO:10) followed by sequencing with primer Lg5 5'-CAC CGC CAA ACT GAT TGT-3' (SEQ ID NO:11) to confirm the *lgtG* "fixed" mutation had replaced the *lgtG::kan* allele. The resulting strain  $\phi$ 3lgtA2GlgtGL2+ has the genotype: MC58 parent strain; *siaD::ery lgtAG2 lgtG* "fixed".